Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 1821–1825, April 1983
Biochemistry

The sequence $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ forms left-handed Z-DNA in negatively supercoiled plasmids

(Z-DNA antibodies/filter binding assay/DNA-protein crosslinking/chromatin structure/recombination)

ALFRED NORDHEIM AND ALEXANDER RICH

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Contributed by Alexander Rich, December 22, 1982

ABSTRACT **Z-DNA-specific antibodies have been used to demonstrate the formation of left-handed Z-DNA in sequences of $(dC\text{-}dA)_{32} \cdot (dG\text{-}dT)_{32}$ in negatively supercoiled plasmids. Z-DNA was found to form at physiological negative superhelical densities ($\sigma = -0.05$) in the absence of high salt concentration or chemical modifications. The binding site of the antibody to the DNA sequences was demonstrated by crosslinking experiments. $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ sequences are widespread in eukaryotic DNA, and possible roles for such Z elements in chromatin activation or genome rearrangements are suggested.**

Z-DNA is a left-handed conformation of the double helix favored by base sequences containing alternating purines and pyrimidines (1). The bases alternate in their glycosylic torsion angles between *syn* and *anti*; the *anti* conformation is the more stable one for pyrimidines. The details of the Z-DNA conformation were first observed in single-crystal x-ray diffraction analyses (1), but the conversion from right-handed B-DNA to a high-salt structure had been observed much earlier with the finding that poly(dG-dC) shows a near-inversion of its circular dichroism in high concentrations of NaCl (2). Raman spectral studies of poly(dG-dC) in high- and low-salt solutions and of the Z-DNA crystals demonstrated that the high-salt form of the polymer is Z-DNA (3). Unlike B-DNA, left-handed Z-DNA is highly immunogenic. Antibodies have been produced, both polyclonal (4) and monoclonal (5), which bind specifically to Z-DNA. These have been used to demonstrate the existence of Z-DNA in various biological systems (6, 7).

Of the naturally occurring repetitive DNA sequences containing alternating purines and pyrimidines, one of the commonest forms is that which has C and A bases alternating on one strand and G and T on the other strand, $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$. Sequences of this type have been widely reported in biological systems with n up to 40 (8–17). Recently, hybridization studies with radioactive $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ have revealed that stretches with this sequence are distributed in a large variety of eukaryotic species ranging from yeast and *Drosophila* up through the higher eukaryotes (14). It has been reported that $(dC\text{-}dA)_n \cdot (dG\text{-}dC)_n$ can form Z-DNA in fibers (18). It has been suggested that in a high-salt solution this polymer may also form Z-DNA-like structures as indicated by changes in the circular dichroism (19, 20). However, these changes do not occur as readily with this polymer as with $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$. More recently, it has been shown that covalent modification of $(dC\text{-}dA)_n \cdot (dG\text{-}dC)_n$ by acetylaminofluorene will also induce inversion of the circular dichroism of the polymer at NaCl concentrations of 2 M (21).

These findings suggest that $(dC\text{-}dA)_n \cdot (dG\text{-}dC)_n$ sequences may be induced to form Z-DNA but the conditions used are not physiological states. In biological systems DNA is negatively supercoiled (22). It was shown recently that the free energy of plasmid negative supercoiling is a powerful inducer of B-to-Z DNA conformational transitions. This has been demonstrated in circular plasmids by changes in hydrodynamic parameters of sedimentation (23), electrophoretic mobility (23, 24), and nuclease sensitivity (24) and also by the fact that antibodies specific for Z-DNA bind to segments of alternating purine and pyrimidine residues in negatively supercoiled plasmids (25).

In this report, we show that antibodies specific to Z-DNA combine with a negatively supercoiled plasmid containing 64 bases of $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ on the segment containing these residues. Furthermore, this plasmid will form Z-DNA at negative superhelical densities in the physiological range (22).

MATERIALS AND METHODS

Construction of pAN064. The cloning strategy for assembling pAN064 is described in *Results* and outlined in Fig. 1. The $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ sequence we used was originally isolated by Nishioka and Leder (10) from a mouse κ (K2) immunoglobulin light chain gene. It was kindly supplied to us by Philip Leder in the form of plasmid K2A (10), a pBR322–K2 gene hybrid. K2A and pBR322 DNAs were restricted with endonucleases *Acc* I and *Cla* I (New England BioLabs), respectively, and the overhanging ends generated by the cleavage were filled in with DNA polymerase (Klenow fragment, Boehringer Mannheim) in the presence of 1 mM XTPs. After phenol extraction and precipitation in ethanol, the resuspended DNAs were cleaved with *Eco*RI. Cleavage products were separated on a 5% acrylamide gel and the fragments were eluted out of the gel. The pBR322 DNA and the 250-base-pair (bp) fragment derived from K2A (Fig. 1) were ligated with T4 ligase (New England BioLabs) and transformed into *Escherichia coli* bacteria. The cloning was confirmed by restriction endonuclease mapping of transformants.

Plasmid Preparation, Agarose Gel Analysis of Negative Supercoiling, Antibody-Preparation, Formation of Antibody–Plasmid Complexes, and Antibody—Plasmid Crosslinking. These were performed as described (25). Topoisomerase I (from calf thymus) was obtained from Bethesda Research Laboratories.

RESULTS

Plasmid pANO64 is a pBR322 hybrid plasmid containing a 250-bp segment that was cleaved out of the 3′ noncoding region of the K2 mouse immunoglobulin κ light chain variable region by using *Eco*RI and *Acc* I. This segment contains a $(dC\text{-}dA)_{32} \cdot (dG\text{-}dT)_{32}$ sequence in which one cytosine residue is re-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: bp, base pair(s).

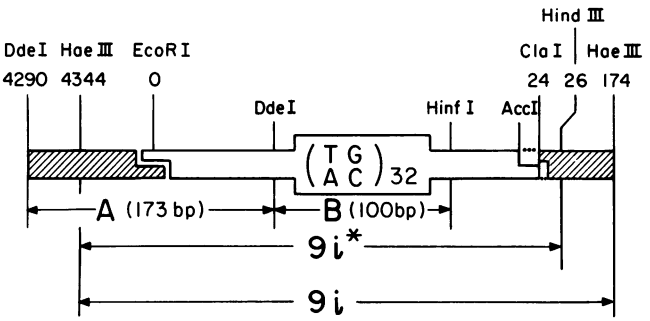

FIG. 1. Diagram of the $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$ segment obtained from a mouse plasmacytoma κ variable region inserted into the plasmid pBR322. The pBR322 sequence is cross-hatched; the insert is open. The insert was ligated into pBR322 *Hae* III restriction fragment 9 between the *Eco*RI site and the *Cla* I sites. Other restriction endonuclease cleavage sites are indicated. The numbers beneath the restriction endonucleases refer to the position of the cleavage sites in the pBR322 sequence.

placed by a thymidine (10). The blunt *Acc* I end of the K2 segment and the *Cla* I end of pBR322 were ligated together as were the two *Eco*RI ends. Radioactively labeled pANO64 DNA was relaxed by a topoisomerase I reaction in the presence of varying concentrations of the intercalator ethidium bromide. Removal of the ethidium when the topoisomerase is inactivated yields negatively supercoiled plasmids. The negative superhelical density of the plasmid preparations is thus adjusted to desired levels. The actual level of negative supercoiling was analyzed by agarose gel electrophoresis to determine the mean of the Gaussian distribution of topoisomers (26).

Z-DNA was detected in negatively supercoiled plasmids through the use of affinity-purified rabbit antibodies to Z-DNA (6). The antibodies were added to the negatively supercoiled plasmid in a simple filter binding assay. In this assay, the supercoiled plasmid will pass through the nitrocellulose filter if it is not bound to the antibody but will be retained on the filter if it is DNA–antibody complex. Binding assays were carried out at two different salt concentrations.

No binding was found for totally relaxed plasmids (data not shown). At 300 mM ionic strength (Fig. 2*A*), the plasmid preparation with negative superhelical density of $\sigma = -0.055$ was not bound by the antibody. However, when the negative supercoiling was increased above this value the amount of binding increased markedly. At $\sigma = -0.070$, $\approx$70% of the plasmids bound to the filter paper when assayed in 300 mM Na; however, in 150 mM Na (Fig. 2*B*) at a similar negative superhelical density virtually all of the plasmids bound to the filter paper.

The data presented in Fig. 2 at an antibody concentration of 860 nM are replotted in Fig. 3 as a function of negative superhelical density. The midpoint for the binding of anti-Z-DNA antibodies to pANO64 was at a $-\sigma = 0.068$ in 300 mM Na^+ and $-\sigma = 0.057$ at 150 mM Na^+. The sigmoidal form of the binding curve as a function of negative superhelical density is similar to that which has been observed for the binding of plasmids containing inserts of alternating cytosine and guanine residues. In the previous experiments (25), we demonstrated that negatively supercoiled plasmids with inserts containing these alternating residues form Z-DNA upon negative supercoiling. However, these same experiments showed that the vector pBR322 by itself would also form Z-DNA at a somewhat higher negative superhelical density. Accordingly, it was important to demonstrate that the segment of pANO64 to which the antibody was binding actually was the region containing $(dC\text{-}dA)_n\cdot(dG\text{-}dT)_n$.

To identify the segment converting to Z-DNA with increasing negative superhelical density, the antibody was crosslinked to the plasmid with 0.1% glutaraldehyde (25). The complex was digested with restriction endonucleases and passed through a nitrocellulose filter (all the uncomplexed DNA fragments pass through the filter whereas those crosslinked to the antibody are retained). The DNA fragments in the filtrate were analyzed by acrylamide gel electrophoresis. Because the insert was cloned into pBR322 *Hae* III fragment 9, this now is of approximately the same size as *Hae* III fragment 5 (9i in Fig. 1). However, *Hin*dIII cleavage of fragment 9i generates 9i* which bands between *Hae* III fragments 5 and 6 in a gel electrophoretic separation. *Hae* III/*Hin*dIII double digestions of pANO64 crosslinked in the absence or presence of antibody were compared. After crosslinking in the presence of antibody and passage of the digest through a filter (Fig. 4*B*), peak 9i* was considerably decreased compared to the control

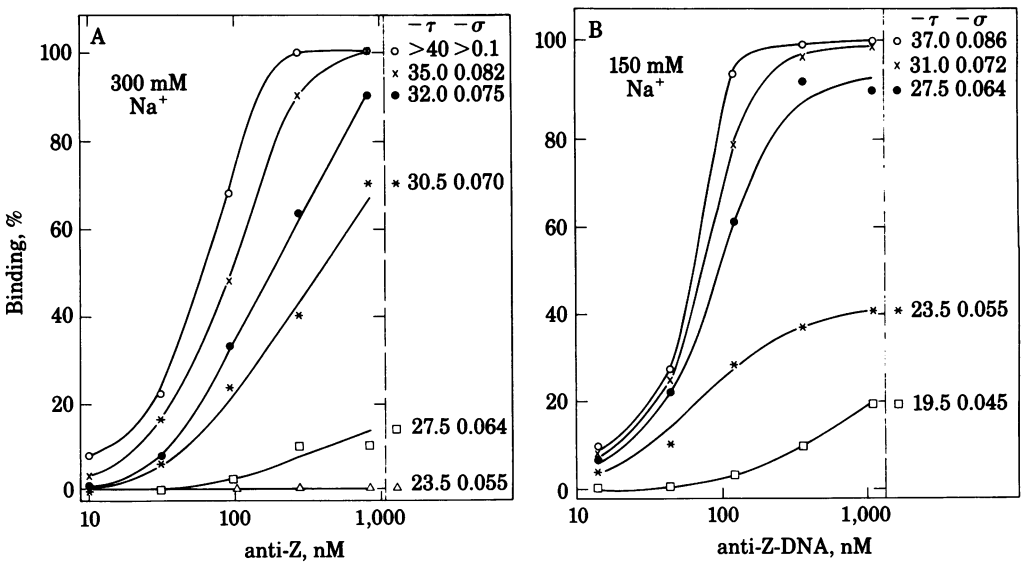

FIG. 2. Binding of radioactive negatively supercoiled plasmids to nitrocellulose filters as a function of antibody concentration for different levels of negative supercoiling ($-\tau$) or negative superhelical density ($-\sigma$). The results are presented at Na^+ concentrations of 300 mM (*A*) and 150 mM (*B*). The binding increased as the negative supercoiling increased above a threshold value.

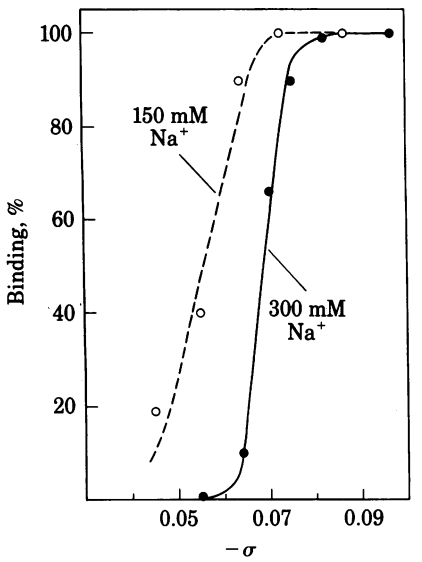

FIG. 3. Binding of plasmid pANO64 to nitrocellulose filters at an antibody concentration of 860 nM as a function of negative superhelicity for two different concentrations of Na^+. A higher negative superhelical density was required for the binding of plasmids at the higher concentration of Na^+.

(Fig. 4A) in the absence of the antibody. This identified the region combining with the antibody within the 288 bp of fragment 9i*.

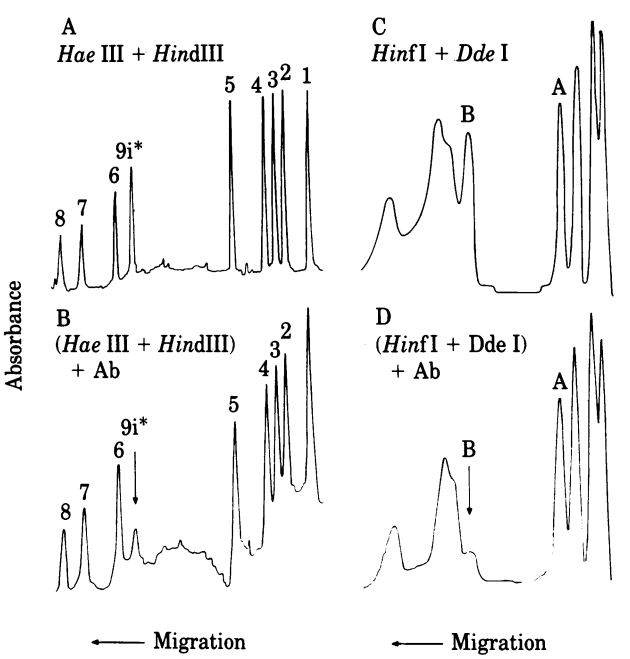

FIG. 4. Densitometer scans of gel electrophoresis patterns of restriction fragments of pANO64 plasmids containing the $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$ sequence. The insert was cloned into *Hae* III fragment 9 which was shortened by digestion with *Hin*dIII to yield fragment 9i* (Fig. 1). (*A* and *B*) *Hae* III/*Hin*dIII DNA fragments present in filtrates after crosslinking of plasmid DNA in the absence (*A*) or presence (*B*) of antibody. The intensity of fragment 9i* is significantly less in *B*, indicating that it has been bound to the nitrocellulose filter. (*C*) Part of the densitometer tracing of the pattern when the plasmid was crosslinked in the absence of antibody and digested with *Hin*FI and *Dde* I. The fragments labeled A and B refer to the 173-bp and 100-bp fragments labeled A and B, respectively, in Fig. 1. (*D*) When the antibody was added to the negatively supercoiled plasmid and crosslinked prior to digestion by restriction enzymes and passage through a nitrocellulose filter, band A was not decreased in intensity but band B was. Two-thirds of the nucleotides in band B were $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$.

Two restriction endonuclease recognition sites straddle the $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$ segment (Fig. 1), a *Hin*fI site and a *Dde* I site separated by 100 bp, of which 64 bp contain $(dC\text{-}dA)_n\cdot(dG\text{-}dT)_n$. Therefore, crosslinked complexes of supercoiled pANO64 and anti-Z-DNA antibody were subjected to *Hin*fI and *Dde* I digestion, and the products were analyzed as described above. The control (Fig. 4C) exhibited a number of restriction fragments including a 170-bp *Dde* I segment designated fragment A (Fig. 1) and the *Hin*fI/*Dde* I segment carrying $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$ designated fragment B. Addition of the antibody produced a marked decrease in fragment B but no appreciable change in any of the other restriction fragments. This experiment demonstrated that the antibody was combining with the 100-bp segment, two-thirds of which consisted of the alternating purine and pyrimidine segment. We thus conclude that the antibody is recognizing Z-DNA in $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$ rather than in another segment of alternating purines and pyrimidines. All of these crosslinking experiments were carried out with plasmid preparations at a relatively high negative superhelical density ($-\sigma = 0.086$) in order to provide a better opportunity for other potential segments to adopt the Z-DNA conformation. Despite this, no other restriction fragments were decreased in intensity.

DISCUSSION

The data presented above show that plasmids containing the $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$ element will form left-handed Z-DNA at negative superhelical densities above $\sigma = -0.05$. A number of additional factors are known to stabilize Z-DNA, including increased cation concentration (2), methylation of C-G sequences (27), and possibly proteins that bind specifically to Z-DNA (28). However, in the present experiments the $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$ segment formed Z-DNA in the absence of excess salt, chemical modifications, or Z-DNA binding proteins. The fact that this conversion occurs at physiological negative superhelical densities suggests the possibility that negative supercoiling may be a major driving force in forming Z-DNA *in vivo*.

In the experiments described here we used the specificity of antibody binding to Z-DNA to demonstrate formation of the left-handed double helix. A number of experiments that illustrate the specificity of these affinity-purified polyclonal antibodies for Z-DNA have been carried out (4, 6). The immunogen used for forming these antibodies was a Z-DNA form of $(dC\text{-}dG)_n\cdot(dG\text{-}dC)_n$ which nonetheless gives rise to an antibody which recognizes the $d(CA/GT)_n$ form of Z-DNA. Monoclonal antibodies have been developed against Z-DNA, and different monoclonal antibodies interact with different parts of the double helix (5). Z-DNA is found in a family of molecules and the form adopted by the plasmid segments containing $d(CA/GT)_n$ is one member of that family. Further characterization (unpublished data) has demonstrated that some monoclonal antibodies will recognize this member of the Z-DNA family whereas others do not.

In earlier experiments the formation of Z-DNA in plasmid inserts containing $(dC\text{-}dG)_n\cdot(dG\text{-}dC)_n$ sequences was studied as a function of negative superhelical density (25). The results of that investigation are presented in Table 1 together with the information obtained from Fig. 3 for the median negative superhelical densities at which the different types of alternating purine and pyrimidine segments flipped over to Z-DNA. All of the data in Table 1 were obtained in experiments assayed at 300 mM Na^+. The earlier experiments showed an inverse correlation between the lengths of alternating C-G segments and negative superhelical densities required for their B-to-Z transitions. Furthermore, alternating C-G sequences

Table 1. Median negative superhelical density at which various plasmid segments form Z-DNA

Plasmid	Z-DNA segment	$-\sigma_M$ (300 mM Na^+)
pBR322	$(dPy\text{-}dPu)_7\cdot(dPu\text{-}dPy)_7$*	0.091
pLP014	$(dC\text{-}dG)_7\cdot(dG\text{-}dC)_7$	0.071
pANO64	$(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$†	0.066
pLP32	$(dC\text{-}dG)_{16}\cdot(dG\text{-}dC)_{16}$	0.046

*The pBR322 segment that forms Z-DNA consists of 14 bp of alternating purine and pyrimidine residues with 1 bp out of alternation.
†The 64 bp of $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$ residues in pANO64 include one cytosine that is replaced by a thymidine.

formed Z-DNA more readily at a given superhelical density than did a segment of pBR322 DNA that also contained additional A·T bp. As shown in Table 1, the 64-bp segment of alternating C-A and G-T residues required a higher negative superhelical density than the 32-bp segment of alternating C-G and G-C residues. This provides further evidence that A·T base pairs form Z-DNA less readily than do C·G base pairs. The probable reason for this is the three-dimensional structure of Z-DNA (1) in which a bridging water molecule is hydrogen bonded to the amino group on the 2 position of guanine and to the phosphate on the 3 position of the sugar. This bridging water molecule stabilizes guanosine in the *syn* conformation, and the absence of an analogous amino group in adenine means that the bridging water molecule is absent.

Fig. 3 shows a sharp rise in plasmid binding as a function of negative superhelical density near the median value of $-\sigma = 0.066$ at 300 mM Na^+. This indicates cooperativity in the B-to-Z transition, suggesting that the entire segment of alternating purine and pyrimidine bases may be flipping from B- to Z-DNA when the critical negative superhelical density is reached. It has been shown previously that at $-\sigma \approx 0.09$ a segment of the natural sequence of pBR322 forms Z-DNA (25). The experiments shown in Fig. 4 demonstrate that there is no crosslinking of the antibody to that segment of pBR322 at the negative superhelical densities at which the antibody crosslinks to the segment of $(dC\text{-}dA)_{32}\cdot(dG\text{-}dT)_{32}$. In pANO64 there are 64 bp of alternating purine and pyrimidine. If they all are converted from a right-handed to a left-handed double helix, approximately 11 negative superhelical turns are removed from the plasmid. The resulting lowering of the negative superhelical density by 0.025 would then make it less likely that any other segments would also flip into Z-DNA. When antibody binding to the plasmids was assayed at 150 mM Na^+, Z-DNA is formed at a lower negative superhelical density ($-\sigma = 0.055$). At first this seems paradoxical because Z-DNA is stabilized at high salt concentrations. This phenomenon has already been pointed out elsewhere (24).

The widespread adoption of nucleotide DNA sequence determination techniques has led to the discovery of many examples of natural sequences containing $d(CA/GT)_n$. The sequences listed in Table 2 show that different lengths are found, with n ranging from 4 to 40 (8–17). A number of genes show several clusters of these sequences, and some have imbedded within them sequences with alternating cytosine and guanosine residues which have an even stronger tendency to form Z-DNA than the $d(CA/GT)_n$ sequences (15). We have examined the surrounding sequences of the $d(CA/GT)_n$ segments. Table 2 shows that there does not seem to be any rule that describes the neighboring sequences. If the neighboring sequences represent areas that form transition structures between B-DNA and Z-DNA, there appear to be no obvious nucleotide sequence requirements for such structures.

The overall frequency in occurrence of $d(CA/GT)_n$ sequences in eukaryotic genomes has been examined by Hamada *et al.* (14) by hybridization analysis. The human genome was found to have 50,000 regions in the haploid genome at least 50 bp long; yeast has 100. In a similar way, positive results were found with *Xenopus*, salmon, *Drosophila*, chicken, mouse, and calf. However, no hybridization was found with the *E. coli* genome. Hybridization experiments by Miesfeld *et al.* (12) using a plasmid containing a 34-bp segment of $d(CA/GT)_n$ sequence also showed a high copy number of these elements in *Xenopus*, pigeon, slime mold, yeast, and man. If that hybridization also was due to the same sequences, more than a dozen eukaryotic chromosomes have been shown to have significant numbers of $(dC\text{-}dA)\cdot(dG\text{-}dT)$ segments. As Hamada *et al.* (14) pointed out, $d(CA/GT)_n$ segments represent a type of interspersed middle repetitive DNA widely distributed in eukaryotic chromosomes.

We have reported (28) that proteins with the ability to bind Z-DNA selectively can be isolated from *Drosophila melanogaster* nuclei. These proteins are also able to bind negatively supercoiled plasmids containing $d(CA/GT)_n$ but not linear or relaxed plasmids. In this regard it is interesting that Hamada *et al.* (14) have shown that the *Drosophila* haploid genome has 2,000 copies of a $d(CA/GT)_n$ element with 50 bp or greater.

The data presented here demonstrate that elements of naturally occurring simple sequence DNA with alternating purine and pyrimidine residues can form Z-DNA under negative supercoiling conditions comparable to those found *in vivo* (22). The existence of specific binding proteins further enhances

Table 2. Nucleotide sequences surrounding different elements containing $(dC\text{-}dA)\cdot(dG\text{-}dT)$

Sequence	Genome location	Ref.
ATGG - - - - - - - $(TG)_{40}$ - - - - - - - - - - - - GTTG*	Immunoglobulin intron, $C_H(\mu,\delta)$, mouse	8
CCTC - - - - - - - $(TG)_{38}$ - - - - - - - - - - - - TAAT†	Immunoglobulin intron, V_H, mouse	9
GATA - - - - - - - $(TG)_{32}$ - - - - - - - - - - - - GGAC‡	3′ noncoding, immunoglobulin κ U-V gene, mouse	10
GTGA - - - - - - - $(TG)_{25}$ - - - - - - - - - - - - ACTC	Intron, actin gene, human	11
GACT - - - - - - - $(TG)_{17}$ - - - - - - - - - - - - TTGT	Intergenic σ-β globin, human	12
AAGG - - - - - - - $(TG)_{15}$ - - - - - - - - - - - - ACAG	Intergenic α-globin cluster, human	13
TTCG - - - - - - - $(TG)_{15}$ - - - - - - - - - - - - TAGA	Actin region, human	14
GCTC - - - - - $(TG)_{11}(CG)_3(TG)_9$ - - - - - - - - AGAG	Intron ^{G}γ-globin, human	15
GCTC - - - - - - - $(TG)_{13}$ - - - - - - - - - - - - TCAG	Intron ^{A}γ-globin, human	15
GCTC - - - - - $(TG)_9(CG)_5(TG)_8$ - - - - - - - - - TCAG	Intron ^{A}γ-globin, human	15
TGCC - - - - - - - $(TG)_{12}$ - - - - - - - - - - - - GTTG	Simian virus 40 integration site into rat	16
GCC - - - - - - - - $(TG)_4$ - - - - - - - - - - - - CCTG	Duplication sites, α-globin, human	17

*One exchange, T to C.
†One exchange, T to C; three exchanges, G to A.
‡One exchange, C to T.

the possibility that $d(CA/GT)_n$ segments may be involved in some aspect of the normal physiological activity of DNA. At present, we cannot make firm statements about what these activities may be. However, analysis of the data of Hamada *et al.* (14) and Miesfeld *et al.* (12) suggests an interesting possibility. If one correlates the copy number of $d(CA/GT)_n$ elements per haploid genome, the existence of one such element per 50–100 kilobases is indicated. DNA of the eukaryotic genome is believed to be organized in domains (29)—i.e., in loops in which the DNA is attached periodically to a nuclear matrix. It is believed that this attachment makes the loops independent superhelical units. The DNA loops or domains are estimated to be in a range of 50–100 kilobases. The agreement in the estimated copy number of $d(CA/GT)_n$ segments and the number of DNA domains in eukaryotic genomes may suggest a functional correlation with a large Z element per domain. DNA conformational transitions of such elements from B-DNA to Z-DNA and vice versa would affect the superhelical density within a topologically constrained domain. The flipping of a segment of 50 bp (or greater) in length would cause a change in 10 (or more) superhelical turns. The present evidence suggests that domains can be either available for transcription (active) or unavailable for transcription (inactive). We might ask whether Z-DNA in the domain is responsible for this activation by influencing the complex higher-order chromatin structure. Is it possible that transitions from Z-DNA to B-DNA (or vice versa) trigger chromatin domain activation? We do not have enough information at present to be able to evaluate this suggestion. It should be noted that the $d(CA/GT)_n$ sequences are not methylated because they do not contain C-G sequences, and they are not able to form a cruciform structure which could be formed with the sequence $(dC\text{-}dG)_n \cdot (dG\text{-}dC)_n$.

Slightom *et al.* (15) suggested that a $d(CA/GT)_n$ sequence may be active in intergenic DNA exchanges in the human fetal γ-globin system. Proudfoot and Maniatis (17) had observed earlier that gene duplication events in the human α-globin system had occurred at a DNA sequence of G-C-C-T-G-T-G-T-G-T-G-C-C-T-G, containing $(dC\text{-}dA)_4 \cdot (dG\text{-}dT)_4$. In this respect it is of interest that the duplicated 72-bp segment of the simian virus 40 is flanked by the sequence A-T-G-T-G-T-G-T. The integration site of simian virus 40 into the rat genome characterized by Stringer (16) also involved a recombinational event at a $d(CA/GT)_n$ sequence. These examples suggest that conformational flexibility of $d(CA/GT)_n$ sequences could be instrumental in DNA rearrangements that are brought about by the specific action of recombinatory proteins. This hypothesis could explain the existence of short sequences of alternating purine and pyrimidine sequences at DNA binding sites of the transposon Tn3 resolvase protein (30). Furthermore, we are intrigued by the C-A-C-T-G-T-G sequence, which is found in the conserved recombinational signals of immunoglobulin D/J segments (31). DNA is normally in a conformational equilibrium between right-handed B-DNA and left-handed Z-DNA. One of the characteristics of a sequence of nucleotides such as $d(CA/GT)_n$ which can form left-handed Z-DNA more easily is the fact that there is always a significant number of these sequences which exist in an untwisted version, neither right-handed nor left-handed. In that form these strands may be able to open up and separate and may be favored to recombine with other similar open strands. Clearly, the possibility of alternative DNA conformations as signals for DNA recombination and genome rearrangements warrants further analysis.

The experiments described here emphasize the conformational potentials inherent in specialized sequences containing alternating purine and pyrimidine residues. Because these sequences can form Z-DNA as well as B-DNA under conditions of negative supercoiling which are found *in vivo*, we must bear in mind the possibility that they may play a significant role in the organization of chromatin as well as in the phenomenon of genome rearrangement which is constantly occurring in evolving biological systems.

We thank Drs. B. D. Stollar and E. M. Lafer for supplying us with affinity-purified antibodies and J. Brosius for advice in the DNA cloning experiment. We also thank S. Almo for enthusiastic assistance. A.N. was supported by a fellowship from the Charles A. King Trust (Boston, MA). This research was supported by grants from the National Institutes of Health, the American Cancer Society, and the National Aeronautics and Space Administration.

1. Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. & Rich, A. (1979) *Nature (London)* **287**, 680–686.
2. Pohl, F. M. & Jovin, T. M. (1972) *J. Mol. Biol.* **67**, 375–396.
3. Thamann, T. J., Lord, R. C., Wang, A. H.-J. & Rich, A. (1981) *Nucleic Acids Res.* **9**, 5443–5457.
4. Lafer, E. M., Möller, A., Nordheim, A., Stollar, B. D. & Rich, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3546–3550.
5. Möller, A., Gabriels, J. E., Lafer, E. M., Nordheim, A., Rich, A. & Stollar, B. D. (1982) *J. Biol. Chem.* **257**, 12081–12085.
6. Nordheim, A., Pardue, M. L., Lafer, E. M., Möller, A., Stollar, B. D. & Rich, A. (1981) *Nature (London)* **294**, 417–422.
7. Lipps, H. J., Nordheim, A., Lafer, E. M., Ammermann, D., Stollar, B. D. & Rich, A. (1983) *Cell*, in press.
8. Lin, C.-P., Tucker, P. W., Mushinski, J. F. & Blattner, F. R. (1980) *Science* **209**, 1348–1353.
9. Kim, S., Davis, M., Sinn, E., Patten, P. & Hood, L. (1981) *Cell* **27**, 573–581.
10. Nishioka, Y. & Leder, P. (1980) *J. Biol. Chem.* **255**, 3691–3694.
11. Hamada, H. & Kakunaga, T. (1981) *Nature (London)* **298**, 396–398.
12. Miesfeld, R., Krysstal, M. & Arnheim, N. (1980) *Nucleic Acids Res.* **9**, 5931–5937.
13. Hess, J. F., Fox, M., Schmid, C. W. & Shen, C.-K. J. (1983) *Cell*, in press.
14. Hamada, H., Petrino, M. G. & Kakunaga, T. (1982) *Proc. Natl. Acad. Sci. USA* 6465–6469.
15. Slightom, J. L., Blechl, A. E. & Smithies, O. (1980) *Cell* **21**, 627–638.
16. Stringer, J. R. (1982) *Nature (London)* **296**, 363–366.
17. Proudfoot, N. J. & Maniatis, T. (1980) *Cell* **21**, 537–544.
18. Arnott, S., Chandrasekaran, R., Birdsall, D. L., Leslie, A. G. W. & Ratliff, R. L. (1980) *Nature (London)* **283**, 743–745.
19. Vorlickova, M., Kypr, J., Stokrova, S. & Sponar, J. (1982) *Nucleic Acids Res.* **10**, 1071–1080.
20. Zimmer, C., Tymen, S., Marck, C. & Guschlbauer, W. (1982) *Nucleic Acids Res.* **10**, 1081–1091.
21. Wells, R. D., Miglietta, J. J., Klysik, J., Larson, J. E., Stirdivant, S. M. & Zacharias, W. (1982) *J. Biol. Chem.* **257**, 10166–10171.
22. Bauer, W. R. (1978) *Annu. Rev. Biophys. Bioeng.* **7**, 287–313.
23. Peck, L. J., Nordheim, A., Rich, A. & Wang, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4560–4564.
24. Singleton, C. K., Klysik, J., Stirdivant, S. M. & Wells, R. D. (1982) *Nature (London)* **299**, 312–316.
25. Nordheim, A., Lafer, E. M., Peck, L. J., Wang, J. C., Stollar, B. D. & Rich, A. (1982) *Cell* **31**, 309–318.
26. Keller, W. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4876–4880.
27. Behe, M. & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1619–1623.
28. Nordheim, A., Tesser, P., Azorin, F., Kwon, Y. H., Möller, A. & Rich, A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7729–7733.
29. Paulson, J. R. & Laemmli, U. K. (1977) *Cell* **12**, 817–828.
30. Grindley, N. D. F., Lauth, M. R., Wells, R. G., Wityk, R. J., Slavo, J. J. & Reed, R. R. (1982) *Cell* **30**, 19–27.
31. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. (1980) *Nature (London)* **286**, 676–683.